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Research Article

Molecular Characterization of Plasmid-mediated Quinolone Resistance in Gram-negative Bacilli from Semen Samples in Ouagadougou

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Abstract

The widespread use of fluoroquinolones has led to the emergence of resistant strains, complicating the treatment of bacterial infections. This study aimed to analyze quinolone resistance in Gram-negative bacilli isolated from semen samples in Burkina Faso. A total of 311 semen samples were used in the study. The bacteria present were isolated and identified using standard methods. Antibiotic susceptibility was assessed, and isolates resistant to at least one of the quinolones tested were analyzed by conventional PCR to detect the resistance genes aac(6')-Ib, qnrA, qnrB, and qnrS. A total of 8 samples (2.58%) were culture-positive, with a predominance of Escherichia coli (62.5%) and Klebsiella pneumoniae (37.5%). All Klebsiella pneumoniae species were susceptible to antibiotics, while Escherichia coli strains showed resistance rates of 50% to ciprofloxacin, 37.5% to norfloxacin, and 12.5% to levofloxacin. Molecular analysis of the isolates revealed a high prevalence of the qnrA gene (75%), followed by the aac(6')-Ib and qnrB genes (50% each). In addition, 50% of isolates contained both the qnrA and qnrB genes, and 25% contained both aac(6')-Ib and qnrA. The detection of these plasmid resistance genes highlights the importance of monitoring the evolution of antibiotic resistance and promoting the judicious use of antibiotics in order to limit its spread.

Keywords

Quinolone Resistance, Gram-negative Bacilli, Semen, PCR, Burkina Faso

1. Introduction

Gram-negative bacilli are involved in various human infections, particularly those affecting the urinary, respiratory, and genital systems. Their ability to develop resistance to antibiotics represents a major challenge [1]. Among the

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available therapeutic alternatives, quinolones play an essential role due to their broad spectrum of activity and efficacy. However, the increase in quinolone-resistant strains over the last few decades poses a significant risk to their clinical use [2]. The main factor contributing to this resistance in Gram-negative bacilli is linked to mutations in the genes responsible for gyrase (gyrA gene) and topoisomerase IV (parC) [3, 4]. In addition, the acquisition of plasmid-mediated resistance genes, particularly *qnrA*, *qnrB*, and *qnrS*, exacerbates this resistance problem [5].

In the field of male genital infections, the main pathogens identified include *Escherichia coli* and *Klebsiella pneumoniae*, both of which are associated with decreased fertility [6]. Screening for antibiotic-resistant bacteria in semen samples appears essential for improving the management of male genital infections. It also provides a better understanding of the role of the male genital tract as a reservoir for multidrug-resistant bacteria. While antibiotic resistance is recognized as an urgent global problem, Burkina Faso faces challenges stemming from insufficient resources for microbiological surveillance, leading to a lack of data on the prevalence of resistant strains and the molecular mechanisms contributing to these genital infections [7, 8]. Therefore, this study seeks to clarify the molecular mechanisms responsible for quinolone resistance in Gram-negative bacilli derived from semen samples in Burkina Faso, with the aim of improving understanding of the dynamics of antibiotic resistance in this particular context.

2. Materials and Methods

2.1. Isolation and Identification of Bacterial Species

The semen samples analyzed in this study were collected at the microbiology laboratory of Saint-Caimille Hospital in Ouagadou from patients admitted for semen culture testing between July 2024 and January 2025. Gram-negative bacilli were obtained by inoculating each sample onto Uri Select, CLED, and EMB agar. After incubation for 24 hours at 37 ℃, bacterial species were identified using biochemical tests, including urease and indole, as well as the API 20E panel (BioMerieux), in accordance with the manufacturer's recommendations.

The selected colonies were then cultured on Muller-Hinton agar at 37 ℃ for 24 hours to ensure their purification. After this process, the purified colonies were used for antibiogram analysis.

2.2. Antibiotic Sensitivity Testing

The sensitivity of bacterial strains to antibiotics was assessed by diffusion on Mueller-Hinton (MH) agar, in accordance with the protocols of the French Society for Microbiology's Antibiogram Committee (EUCAST/CA-SFM, 2024). A bacterial suspension was prepared in a tube containing 5 mL of physiological saline, the density of which was adjusted to 0.5 according to the McFarland scale. This suspension was then spread evenly over the surface of the MH agar in close streaks. Disks impregnated with ciprofloxacin (5 µg), norfloxacin (5 µg), ofloxacin (5 µg), and levofloxacin (5 µg) were then placed approximately 2 cm apart using sterile forceps. After incubation for 24 hours at 37 ℃, the diameters of the inhibition zones surrounding the discs were measured and compared to the reference values provided by the CA-SFM. To validate the antibiogram, a strain *of Escherichia coli* ATCC 25922 provided by the microbiology laboratory of the Princess Sarah Clinic was used.

2.3. Molecular Analysis

2.3.1. DNA Extraction

Genomic DNA extraction from bacterial strains was performed by boiling, as detailed by Amana MD, with a slight adjustment [9]. Pure colonies, cultured for 24 hours, were selected and suspended in 200 µL of sterile distilled water, then placed in properly labeled Eppendorf tubes. These tubes were then incubated in a water bath at 100 ℃ for 15 minutes to promote the release of bacterial genetic material.

After the procedure, centrifugation was performed for 10 minutes at 13,000 rpm, allowing the supernatant containing the extracted DNA to be recovered, which was then transferred to a new Eppendorf tube. DNA quantification and purity assessment were performed using NanoDrop. Consequently, any sample with an A260/A280 absorbance ratio between 1.8 and 2 was considered pure.

2.3.2. Amplification of Extracts by Conventional PCR

The DNA extracts were amplified by conventional PCR (polymerase chain reaction) using the GeneAmp PCR System 9700 thermocycler (Applied Biosystems, USA). This approach enabled the detection of the *aac(6)-Ib* resistance gene, as well as the *qnrA*, *qnrB*, and *qnrS* proteins. The reaction mixture, with a total volume of 20 µL, consisted of 4 µL of Firepol® Master Mix 5X, 0.6 µL of sense primer, 0.6 µL of antisense primer, 12.8 µL of PCR water, and 2 µL of bacterial DNA from each strain [10]. A negative control comprising the elution buffer was included. The *qnrA* and *qnrB* genes were detected by multiplex PCR without loss of specificity. Optimizing the hybridization temperature and primer concentrations reduced the risk of non-specific amplification. No signal was observed in the negative controls, suggesting the absence of false positive. Table 1 presents a summary of the primer sequences used for PCR.

Table 1. Primers and amplicon sizes.

Genes	Sequences (5'-3')	Size (bp)	References
aac (6')-Ib	F: TTGCGATGCTCTATGAGTGGCTA R: CTCGAATGCCTGGCGTGTTT	482	[10]
qnr S	F: GCAAGTTCATTGAACAGGGT R: TCTAAACCGTCGAGTTCGGCG	428	[10]
qnr A	F: AGAGGATTTCTCACGCCAGG R: TGCCAGGCACAGATCTTGAC	580	[10]
qnr B	F: GGMATHGAAATTCGCCACTG R: TTTGCYGYYCGCCAGTCGAA	264	[10]

aac(6')-Ib: aminoglycoside acetyltransferase qnr: quinolone resistance protein

The amplification program summarized in Table 2 was used for PCR.

Table 2. PCR program for the detection of resistance genes.

Genes	PCR program	References
Aac(6)-Ib qnrA qnrB	1) 1 cycle of: initial denaturation at 95°C for 15 minutes 2) 30 cycles of: Denaturation at 95°C for 1 minute, Hybridization at 55°C for 1 minute, Elongation at 72°C for 1 minute 3) 1 cycle of: Final extension at 72°C for 5 minutes	[10]
qnr S	1) 1 cycle of: initial denaturation at 95°C for 15 minutes 2) 30 cycles of: Denaturation at 95°C for 1 minute, Hybridization at 56°C for 1 minute, Elongation at 72°C for 1 minute 3) 1 cycle of: Final extension at 72°C for 5 minutes	[10]

2.3.3. Agarose Gel Electrophoresis

The PCR-amplified DNA fragments were separated by electrophoresis on a 1.5% agarose gel prepared in a 1X Tris-acetate-EDTA solution with 8 µL of ethidium bromide added. A 100 bp molecular weight marker was used to estimate the size of the fragments obtained. Migration was performed at a voltage of 100 V for 35 minutes. After migration, the DNA fragments were visualized under UV light using the Vilber E-Box Transilluminator, and the images were recorded.

2.4. Statistical Analysis

The collected data were entered into Excel 2019 software. Antibiotic resistance rates and the prevalence of resistance genes were calculated using SPSS 21 software.

3. Results

3.1. Isolation and Identification

Of the 311 semen samples analyzed, 8 (2.58%) were culture positive. Of these isolates, 62.5% (5/8) were *Escherichia coli* and 37.5% (3/8) were *Klebsiella pneumoniae*.

3.2. Antibiotic Sensitivity Tests

Each *Klebsiella pneumoniae* isolate was found to be sensitive to all antibiotics evaluated (see Table 3). In contrast, *Escherichia coli* isolates showed resistance rates of 50% to ciprofloxacin, 37.5% to norfloxacin, and 12.5% to levofloxacin. However, all isolates were sensitive to ofloxacin.

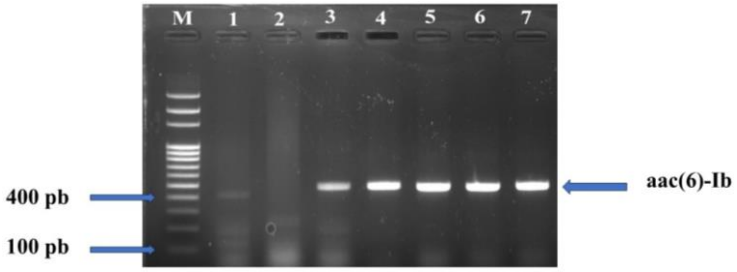
Table 3. Sensitivity of isolates to antibiotics.

Germ	Ciprofloxacin		Levofloxacin		Norfloxacin		Ofloxacin	
	N	%	N		N		N	
E. coli								
S	1	12.5	4	50.0	2	25.0	8	100
R	4	50.0	1	12.5	3	37.5	0	0.0
Klebsiella								
S	3	37.5	3	37.5	3	37.5	0	0.0
R	0	0.0	0	0.0	0	0.0	0	0.0
Total	8	100	8	100	8	100	8	100

S: sensitive R: resistant N: effective

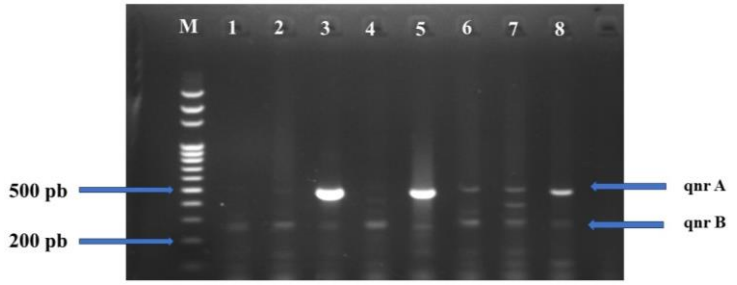
3.3. Characterization of Resistance Genes

The DNA of strains resistant to at least one of the antibiotics tested was analyzed by conventional PCR to detect the presence of the *aac(6')-Ib*, *qnrA*, *qnrB*, and *qnrS* genes. As the primer pairs used were specific to the genes sought, the presence of bands measuring 482 bp, 580 bp and 264 bp was defined as characteristic of the presence of the *aac(6')-Ib* gene (Figure 1), *qnrA* and *qnrB* (Figure 2), respectively.



Legend: M molecular weight marker; 2: absence of the *aac(6')-Ib* gene; 3 to 7: presence of the *aac(6')-Ib* gene

Figure 1. Agarose gel of aac(6')-Ib PCR products.



Legend: M molecular weight marker; 1, 2, and 4: presence of the qnrB gene; 3, 5, 6, 7, and 8: presence of the qnrA and qnrB genes.

Figure 2. Agarose gel of qnr A and qnr B PCR products.

3.4. Frequency of Resistance Genes

Electrophoretic analysis of the PCR products revealed a high prevalence of the *qnrA* gene (75%), followed by the *aac(6')-Ib* and *qnrB* genes (50% each). No isolates carried the *qnrS* gene (Table 4). Regarding gene coexistence, 50% of isolates carried the *qnrA-qnrB* genes, while 25% harbored the *aac(6')-Ib-qnrA* combination. No isolates carried all three

detected genes simultaneously.

Table 4. *Frequency of resistance genes.*

Resistance gene	Present		Absent		Total	
	N	%	N		N	
aac (6)-Ib	2	50.0	2	50.0	4	100
qnr A	3	75.0	1	25.0	4	100
qnr B	2	50.0	2	50.0	4	100
qnr S	0	0.0	4	100	4	100
qnr A-qnr B	2	50.0	2	50.0	4	100
aac (6)-Ib-qnr A	1	25.0	3	75.0	4	100

N: number of individuals aac(6')-Ib: aminoglycoside acetyltransferase qnr: quinolone resistance protein

4. Discussion

In this study, 2.58% (8/311) of the semen samples analyzed were culture-positive, with a prevalence *of Escherichia coli* of 62.5% (5/8) and *Klebsiella pneumoniae* of 37.5% (3/8). These results are consistent with previous studies demonstrating the frequent involvement *of E. coli* in genital and urinary tract infections in men [6]. In addition, research conducted in Tunisia has highlighted the important role of *Escherichia coli* in these infections [11].

Our analyses revealed that all *Klebsiella pneumoniae* strains were sensitive to the antibiotics tested, while *E. coli* strains showed varying levels of resistance to fluoroquinolones. The resistance rates observed were 50% (4/8) for ciprofloxacin, 37.5% (3/8) for norfloxacin, and 12.5% (1/8) for levofloxacin. These resistance rates are consistent with the results of additional research conducted in Burkina Faso, indicating an upward trend in resistance to fluoroquinolones [12].

Escherichia coli resistance to quinolone antibiotics results from a combination of chromosomal mutations, particularly in the gyrA and parC genes. These mechanisms enable the bacteria to reduce the effectiveness of certain quinolone antibiotics by modifying their target sites or limiting their accumulation in the cell [1].

The increase in resistance is significantly affected by overconsumption and, in some cases, misuse of these antibiotics [1, 13]. However, an important finding of our research is that each isolate tested was sensitive to Ofloxacin, potentially due to differences in the mechanisms of resistance related to fluoroquinolones [14].

Genetic analysis of antibiotic-resistant bacteria showed a significant presence of the *qnrA* gene, found in 75% (3/4) of isolates, as well as the *aac(6')-Ib* and *qnrB* genes, each identified in 50% (2/4) of samples. However, the *qnrS* gene was absent from all isolates. These results are consistent with those reported in Korea, which indicated a high prevalence of *Escherichia coli* isolates possessing both the *qnr* and *aac(6')-Ib-cr* genes [15]. The detection of these genes suggests a potential reduction in the effectiveness of quinolones used in the management of urogenital infections in Burkina Faso. In the absence of a thorough diagnosis, this situation exposes patients to an increased risk of treatment failure. In addition, these resistances could contribute to impaired sperm quality and lead to male infertility.

Our results showed the simultaneous presence of several resistance genes in *E. coli*: 50% had both *qnrA* and *qnrB*, while 25% contained a combination of *aac(6')-Ib* and *qnrA*. This accumulation of genes could increase resistance to fluoroquinolones and promote the transmission of these resistances to other bacterial species [4, 10]. These results highlight the importance of systematic surveillance of antibiotic resistance in bacteria responsible for male genital infections. The repeated detection of resistance genes located on plasmids indicates a significant risk of transfer of these resistances between bacterial populations. Such transmission could ly compromise the effectiveness of fluoroquinolones in the treatment of common bacterial infections [1]. To address this problem, it is essential to limit the excessive use of antibiotics and promote more rigorous methods of prescribing and monitoring bacterial resistance [16].

However, this study has certain limitations. The small size of the Gram-negative bacilli and the limited geographical origin of the isolates do not allow the results to be generalized. In addition, the molecular analysis did not explore all possible resistance mechanisms and focused on a specific panel of quinolone resistance genes.

Despite these limitations, this study provides original and relevant data on quinolone resistance in male genital infections in Burkina Faso.

These results offer various avenues for further research. A relevant first step would be to expand the study to include a larger number of samples and to incorporate an analysis of the genetic mutations contributing to quinolone resistance. In addition, research on the transmission of these resistance genes within the Burkinabe population would enable more effective targeting of strategies to prevent and control antibiotic resistance.

5. Conclusion

This study examined the characteristics of quinolone resistance in Gram-negative bacilli isolated from semen samples at Saint Camille Hospital in Ouagadougou. Although the positive culture rate was relatively low (2.58%), the presence of *Escherichia coli* and *Klebsiella pneumoniae* in these samples highlights the importance of microbiological surveillance of infections of the male genital tract. Analysis of antibiotic susceptibility profiles revealed a notable level of resistance to

fluoroquinolones among *Escherichia coli* strains, particularly with regard to ciprofloxacin (50%) and norfloxacin (37.5%). Identification of resistance genes indicated a high prevalence of the *qnrA* gene (75%), with the *aac(6')-Ib* and *qnrB* genes each present at 50%, suggesting that the spread of resistance may be partially supported by mobile genetic elements. The coexistence of multiple resistance genes in some isolates reinforces the need for increased monitoring of quinolone resistance mechanisms in the region.

Abbreviations

Aac	Aminoglycoside Acetyltransferase
Qnr	Quinolone Resistance Protein
EMB	Eosin Methylene Blue
MH	Muller Hinton
PCR	Polymerase Chain Reaction
CLED	Cystine Lactose Electrolyte Deficient
SS	Salmonella-Shigella
DNA	Deoxyribonucleic Acid
EUCAST	European Committee on Antimicrobial Susceptibility Testing
CASFM	Antibiogram Committee of the French Society of Microbiology

Author Contributions

Olawoumi Fabrice Kouta: Conceptualization, Formal Analysis, Investigation, Methodology, Writing – original draft

Amana Metuor Dabire: Conceptualization, Formal Analysis, Investigation, Methodology, Supervision, Validation, Visualization

Rabietou Nikiema: Formal Analysis, Investigation, Methodology

Lionel Eliada Benoit Bambara: Formal Analysis, Investigation, Methodology

Jacques Simpore: Conceptualization, Formal Analysis, Methodology, Project Administration, Supervision, Validation

Conflicts of Interest

The authors have declared no conflicts of interest.

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